

## Hepatitis Virus Vaccines: Present Status

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During the past decade there has been extraordinary progress toward the development of vaccines for the prevention of type A and type B hepatitis.

The successful propagation of hepatitis A virus in cell culture in 1979 was followed by the preparation of experimental live attenuated hepatitis A vaccines that have been shown to induce antibody in marmosets and chimpanzees and protect immunized marmosets against challenge with hepatitis A virus. The first human immunization trials will begin in mid-1982.

An inactivated hepatitis B vaccine that was licensed in the United States in November 1981 has been shown to be safe, immunogenic, and effective. When this vaccine becomes available for use in July 1982, it will be recommended for persons who are considered to be at increased risk of contracting hepatitis B infection. Future generations of hepatitis B vaccines may be prepared from hepatitis B surface antigen derived from DNA recombinant technology or by *in vitro* synthesis of HBs Ag determinants by chemical means.

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It was in 1947, 35 years ago, that Dorothy Horstmann and her colleagues described two institutional outbreaks of infectious hepatitis, a disease that was formerly called "acute catarrhal jaundice" [1]. Her study demonstrated that the disease was relatively mild and of short duration in children as compared with adults. During that same year MacCallum of Great Britain proposed that the term "type A hepatitis" be designated for infectious hepatitis and "type B hepatitis" for serum hepatitis.

Studies by various investigators during the 1940s and 1950s provided convincing evidence of the existence of two immunologically distinct agents: hepatitis A virus (HAV) and hepatitis B virus (HBV). However, all efforts to identify these agents or to transmit them to animals were unsuccessful. In addition, attempts to propagate these viruses in cell culture also ended in failure during the 1950s and 1960s. In spite of these obstacles, progress toward the development of hepatitis A and B vaccines is very encouraging. The first human trials with an experimental live attenuated hepatitis A vaccine will begin in 1982. In addition, an inactivated hepatitis B vaccine that was licensed in November 1981 will be available for use by July 1982.

The importance of hepatitis A and B as worldwide problems has been well recognized for many years. These infections are major causes of acute and chronic liver disease in developing and developed countries of the world.

### VIRAL HEPATITIS IN DEVELOPING COUNTRIES

In general, type A hepatitis is a relatively benign disease in developing countries; it usually occurs as an inapparent infection or mild illness, predominantly in infants

and children. Complications are rare, recovery is usually complete, and most adults are immune. Consequently, there is no urgent need for a hepatitis A vaccine in these areas.

In contrast, type B hepatitis is a more serious infection in children as well as adults; it may be complicated by a chronic hepatitis B carrier state and by chronic active hepatitis that may progress to cirrhosis and primary hepatocellular carcinoma (PHC). The hepatitis B carrier rate in various Asian and African countries has ranged between 10 percent and 20 percent, an indication of the severity and extent of chronic liver disease in these areas. In these countries PHC is the most common type of cancer, accounting for 20 percent to 40 percent of all malignant tumors. Consequently, under the conditions existing in these countries, there is an urgent need for an effective hepatitis B vaccine.

### VIRAL HEPATITIS IN DEVELOPED COUNTRIES

The situation is strikingly different in most developed countries where improvements in sanitation and hygiene have been associated with a declining incidence of hepatitis A infection, especially in children. This phenomenon is responsible for the corresponding increase in susceptibility of the adult population. For example, today more than 60 percent of healthy adults in the United States are susceptible to type A hepatitis. Since type A hepatitis is a moderately severe disease in adults, there may be a need for a hepatitis A vaccine to protect those who travel to highly endemic areas or are exposed to immigrants from endemic areas, especially in schools, hospitals, refugee camps, institutions, and other crowded settings. A safe and effective hepatitis B vaccine is needed for high-risk population groups in developed as well as developing countries of the world. Various high-risk groups for whom the vaccine is recommended will be described later.

### HEPATITIS A VACCINE

The sequence of events that led to the development of an experimental live attenuated hepatitis A vaccine began 15 years ago. In 1967 Deinhardt and colleagues [2] reported the successful transmission of human HAV to marmoset monkeys. These observations were subsequently confirmed by Mascoli and colleagues [3] and other investigators. In 1973 Feinstone and colleagues [4] identified 27 nm virus-like particles in stools obtained from patients with acute type A hepatitis. These particles were initially identified by immune electronmicroscopy and later they were characterized as RNA viruses closely related to the enterovirus family of agents [5,6].

The availability of a specific antigen (HA Ag) led to the development of specific tests for the identification of both the virus and its antibody (anti-HAV). These serologic tests enabled investigators to identify susceptible chimpanzees, excellent animals models for hepatitis A vaccine studies.

The crucial contribution that led to the development of a hepatitis A vaccine was the successful propagation of human HAV in cell culture *in vivo* by Provost and Hilleman in 1979 [7]. This important milestone was achieved 30 years after Enders, Weller, and Robbins first cultivated poliovirus in cell culture [8].

Provost and Hilleman cultivated HAV in primary explant cell cultures of marmoset livers and in normal fetal rhesus kidney cell culture (FR K6). The virus was identified by immunofluorescence, immunofluorescence blockade, serum neutralization, immune adherence, radioimmunoassay, immune electronmicroscopy,

copy, and marmoset inoculation tests. No cytopathology was observed. These observations were confirmed and extended by Frösner, Deinhardt, and colleagues [9], and by Purcell and colleagues [10]. The virus has been isolated directly from human feces, and it has been propagated in a human diploid cell line.

During the course of a meeting of the U.S.-Japan hepatitis panel in March 1982, Dr. Maurice Hilleman of Merck Institute for Therapeutic Research stated that "attenuated live virus vaccines have been prepared that induce antibody in marmosets and chimpanzees and protect marmosets against challenge infection." The initial human immunization trials should begin by mid-1982.

### HEPATITIS B VACCINE

The prerequisite for the development of poliovaccine, measles, mumps, rubella, and hepatitis A vaccines was the successful cultivation of these agents in cell culture, thereby providing the huge quantities of antigen required for vaccine production. To date, no one has successfully propagated HBV in cell culture. Nevertheless, it has been possible to develop a safe, immunogenic, and effective inactivated hepatitis B vaccine.

The crucial milestone that led to the development of a hepatitis B vaccine was the discovery of the Australia antigen by Blumberg and colleagues in 1965 [11], and its subsequent association with HBV by Prince in 1968 [12]. In 1970 and 1971 our group reported that heat-inactivated serum containing our MS-2 strain of HBV was immunogenic and partially protective [13]. Vaccine development was accelerated in 1973 when Barker and colleagues [14] successfully transmitted HBV to susceptible chimpanzees. This excellent animal model proved to be valuable for evaluation of safety, immunogenicity, and protective efficacy of the vaccine.

Our initial observation, that it was possible to prepare a crude hepatitis B vaccine by the simple procedure of boiling a 1:10 dilution of MS-2 serum in distilled water, was difficult to explain. Later, it became obvious that this unique, complex virus had a surface component, the hepatitis B surface antigen (HBs Ag) that was immunologically distinct from the DNA containing core component, the hepatitis B core antigen (HBc Ag). During the synthesis of HBV in infected hepatocytes, enormous amounts of excess HBs Ag particles are formed and persist in the blood of persons with chronic hepatitis B infection. The blood of hepatitis B carriers that contains between  $10^{10}$  and  $10^{12}$  HBs Ag particles per ml has proved to be an excellent source of antigen for vaccine production.

In 1975 Purcell and Gerin [15] and Hilleman and colleagues [16] described their initial studies with an inactivated hepatitis B vaccine prepared from purified preparations of HBs Ag. Since 1975 similar vaccines have been developed in France [17], in Holland [18], in Japan, and in China [19]. The procedures involved in the preparation of these subunit vaccines have been described in several publications [15,16]. In general, the steps involve the collection of large quantities of HBs Ag-positive plasma for purification of the HBs Ag particles by ammonium sulfate concentration, isopyknic banding on sodium bromide, rate zonal separation on a sucrose gradient, and digestion with pepsin. A 1:4000 dilution of formaldehyde solution is added to eliminate any potential residual live virus and the final product is formulated in an alum adjuvant. The current licensed Merck vaccine (HEPTA-VAX-B, Merck, Sharp & Dohme) contains 20  $\mu$ g of HBs Ag protein per ml.

The first human trials with the inactivated vaccine were initiated in 1975 after safety, immunogenicity, and efficacy had been demonstrated in chimpanzees.

Studies by various investigators during the subsequent six years culminated in the licensure of inactivated hepatitis B vaccines in France and in the United States in 1981. The inactivated hepatitis B vaccine (HEPTAVAX-B, Merck, Sharp & Dohme) that was licensed for use in the United States was evaluated for safety, immunogenicity, and efficacy in more than 6000 persons.

### SAFETY

Clinical reactions in seronegative recipients of vaccine have been minimal and transient. Mild local soreness at the site of inoculation has been the most common side effect. Systemic reactions have been rare. During the course of placebo-controlled, double-blind, randomized studies the incidence of side effects was essentially the same in vaccine and placebo groups [20]. In general, the vaccine has been extremely well tolerated.

### IMMUNOGENICITY

In most studies optimum immunogenicity has been achieved by two inoculations of inactivated hepatitis B vaccine given one month apart and followed by a booster dose six months after the first dose. The anti-HBs response in immunocompetent children and adults has ranged between 30 percent and 50 percent after the first dose, 75 percent to 90 percent after the second dose, and over 95 percent after the booster dose at six months. The vaccine has also proved to be highly immunogenic for newborn infants [21,22]. Barin and colleagues [23] immunized 26 Senegalese infants with three inoculations of vaccine containing 5  $\mu$ g of HBs Ag per dose. The vaccine, given before the babies were one month old, was well tolerated, and it induced an anti-HBs response in 94.7 percent of infants whose prevaccination serum contained no detectable HBs Ag or anti-HBs.

Vaccine-induced antibody has persisted for the three-year period of observation. If these levels decline in the future, a subsequent booster dose will be required. It is anticipated that vaccine-induced anti-HBs should persist for at least five years in those vaccinees who had a good response.

Unfortunately, immunocompromised dialysis patients have not responded to the vaccine as well as healthy adults. Studies to be published by Szmunn and colleagues have revealed an anti-HBs response of 60 percent to 70 percent after three 40  $\mu$ g doses of heptatitis B vaccine.

### EFFICACY

The efficacy of inactivated hepatitis B vaccine was demonstrated by Szmunn and colleagues [20,24] in a superbly designed and implemented placebo-controlled, randomized clinical trial in a high-risk group of male homosexuals. Of 1083 volunteers, 549 received vaccine and 534 received placebo. The results of the study are summarized in Table 1. As indicated in the report by Szmunn and colleagues [24] "the attack rate of hepatitis B virus infections (excluding conversions of anti-HBc alone) was 3.2% in vaccine recipients compared with 25.6% in placebo recipients ( $p < 0.0001$ ). In those who received all three doses of 40  $\mu$ g each, the protective efficacy rate was 100%. The vaccine protected against acute hepatitis B, asymptomatic infection, and chronic antigenemia." Since 20  $\mu$ g doses of vaccine have been shown to be as immunogenic as 40  $\mu$ g doses [25,26], and since the presence of anti-HBs has been shown to be indicative of protection, it is reasonable to conclude that three 20  $\mu$ g doses of vaccine will be protective.

TABLE 1  
Attack Rates for Various Categories of Trial End Points in Placebo and Vaccine Recipients

End Point	Placebo Group		Vaccine Group		Log-Rank Chi-Square	P Value
	No.	Rate	No.	Rate		
Hepatitis B (ALT* > 90 IU only)	45	18.1	7	1.4	29.66	<0.0001
HBV events with ALT > 45 IU	56	21.7	13	3.4	29.56	<0.0001
All HBsAg-positive events	70	24.4	11	3.0	48.63	<0.0001
All HBV events, excluding conversion to anti-HBc alone	73	27.1	14	3.5	44.36	<0.0001
All HBV events, including anti-HBc conversions	93	35.0	29	7.6	38.77	<0.0001

\*ALT = alanine aminotransferase (SGPT).

Source: Szmunn W et al.: N Engl J Med 303:833, 1980. Reprinted, by permission of the New England Journal of Medicine.

## RECOMMENDATION FOR USE OF HEPATITIS B VACCINE

The vaccine is recommended for the protection of infants, children, and adults who are at a high risk of acquiring type B hepatitis [27]. The incidence of past hepatitis B infection in the high-risk groups listed in Table 2 may range between 20 percent and 90 percent, a rate that is fivefold to 18-fold greater than the incidence in such low-risk persons as healthy blood donors.

The decision to test for susceptibility or immunity before immunization will depend predominantly on cost-benefit factors. It is estimated that three doses of vaccine will cost about 100 dollars. Therefore, it would be important to routinely pretest male homosexuals and residents of institutions where hepatitis B infection is highly endemic. For example, only 30 percent to 40 percent of male homosexuals will be susceptible. Pretesting for presence of past infection will save the substantial cost of immunizing 60 percent to 70 percent of those who are immune. As indicated in Table 2, prevaccination testing is recommended for those groups that are in a very high-risk category; it is optional for those in whom the risk is not as great. It should be noted that studies by Dienstag and colleagues [28] have shown that immunization of HBs Ag-positive carriers or immune persons is not associated with untoward side effects.

## PASSIVE-ACTIVE IMMUNIZATION

The use of combined passive immunization with hepatitis B immune globulin (HBIG) and active immunization with inactivated hepatitis B vaccine has been studied by Szmunn and colleagues [29]. They demonstrated that passively acquired anti-HBs did not inhibit an active immune response to the vaccine. Studies currently in progress have been designed to evaluate the efficacy of passive-active immunization for postexposure prophylaxis in infants born of mothers who are infectious HBV carriers.

TABLE 2  
Recommendations for Use of Inactivated Hepatitis B Vaccine in High-Risk Groups

High-Risk Group	Dose of Vaccine,* $\mu\text{g}$	Prevaccination Tests†
Health care personnel	20	Optional
Physicians and dentists		
Nurses		
Paramedical and parodontal personnel		
Laboratory technicians		
Selected patients in hemodialysis and hematology/oncology units	40	Recommended
Children with thalassemia and hemophilia	10–20	Recommended
Residents (clients) and staff of institutions for the mentally handicapped and their classroom contacts	20	Recommended
Household contacts of carriers	20	Recommended
Classroom contacts of carriers	20	Optional
Homosexually active males	20	Recommended
Prostitutes	20	Recommended
Users of illicit drugs	20	Recommended
Prisoners	20	Optional
Certain military personnel	20	Optional
Infants and young children in high-risk areas	10–20	Optional

\*Two doses at a one-month interval and a third (booster) dose as six months.

†BsAg and anti-HBs or anti-HBc tests.

Source: Krugman S: JAMA 247:2012, 1982. Copyright 1982, American Medical Association.

## FUTURE HEPATITIS B VACCINES

This first generation hepatitis B vaccine will be more expensive than other viral vaccines because of the complex process involved in the purification of HBs Ag prepared from human plasma that is purchased from chronic carriers. If large quantities of HBs Ag can be derived from recombinant DNA technology or *in vitro* synthesis of HBs Ag determinants by chemical means, future generations of vaccine may be less costly. This goal and its eventual achievement will be essential in order to meet the urgent needs of the developing nations of the world.

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